

SCIENTIFIC OPINION

Scientific Opinion on the safety of steviol glycosides for the proposed uses as a food additive¹

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)^{2,3}

European Food Safety Authority (EFSA), Parma, Italy

SUMMARY

Following a request from the European Commission, the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion on the safety of steviol glycosides as a sweetener for use in the food categories specified in the dossiers from the three petitioners.

The steviol glycosides produced by the three petitioners are chemically defined mixtures that comprise not less than 95% stevioside and/or rebaudioside A. Stevioside and/or rebaudioside A are more than 95% of the mixture in two of the products. In the third product, rebaudioside A is the major component of the mixture ($\geq 95\%$) together with other glycosides. In addition, smaller amounts of rebaudiosides B, C, D, E and F, steviolbioside, rubusoside and dulcoside A are present in the final mixtures, as indicated by the petitioners.

The three petitioners proposed that the specifications for the steviol glycosides should comply with the specifications adopted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at the 69th meeting.

The petitioners indicated that all manufacturers use the same basic steps to extract steviol glycosides from the leaves of the *Stevia rebaudiana* Bertoni plant, although there is some variation in the later stages of purification and separation of the glycosides. Steviol glycosides can be identified in foods and beverages by High Performance Liquid Chromatography (HPLC) methods.

Different studies assessed the bulk stability of dry steviol glycosides under various storage conditions and in food matrices over a range of pH values, processing conditions, at both room temperature and elevated temperatures. The photostability of the preparation was examined under dry and aqueous conditions. The Panel notes that in these experiments the extent of degradation of the tested steviol glycoside (rebaudioside A) that occurred ranged from a few percent up to 63% under different storage (pH and temperature) and food production conditions. The Panel notes that in presence of high temperatures (e.g. heating, baking) substantial degradation of steviol glycosides might take place.

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Stevioside as a sweetener was evaluated by the Scientific Committee for Food (SCF) in 1984, 1989 and 1999. The SCF concluded that the use of stevioside was “toxicologically not acceptable” due to insufficient available data to assess its safety.

JECFA reviewed the safety of steviol glycosides (in 2000, 2005, 2006, 2007, and 2009) and established an ADI for steviol glycosides (expressed as steviol equivalents) of 4 mg/kg bw/day.

The Panel evaluated oral animal studies of metabolism and toxicokinetics, laboratory animal toxicity studies, *in vitro* and *in vivo* genotoxicity studies, and human studies with single or repeated administration of steviol glycosides.

Metabolic studies with steviol glycosides in animals and humans demonstrated that intact steviol glycosides are poorly absorbed after oral exposure but that they are hydrolysed by the microflora in the colon to steviol. A large amount of steviol is absorbed; the rest is excreted in the faeces. In the liver, steviol undergoes conjugation with glucuronic acid to form steviol glucuronide. The only inter-species difference is that the glucuronide is excreted primarily *via* the urine in humans and *via* the bile in rats. No accumulation of steviol glycoside derivatives occurs in the body. Besides steviol glucuronide, no other derivatives could be detected in the urine of humans exposed orally to steviol glycosides.

Rebaudioside A and stevioside both show similar pharmacokinetics in the rat. In humans, rebaudioside A and stevioside are also metabolised and excreted by similar pathways. Therefore, the Panel considers the results of toxicological studies on either stevioside or rebaudioside A applicable for the safety assessment of steviol glycosides in general.

In some subchronic and carcinogenicity studies, and also in the 2-generation reproductive toxicity study body weight gains were lower in the treated groups compared to the controls. In these studies decreases in feed consumption and in feed conversion efficiency were recorded. The Panel considers the effects on body weight as not adverse or indicative of toxicity but related to lower palatability and lower nutritional value of feed containing the steviol glycosides. Therefore body weight parameters are not considered to be appropriate endpoints for setting No-Observed-Adverse-Effect Levels (NOAELs) for these studies.

Overall, stevioside and rebaudioside A do not show evidence of genotoxicity *in vitro* or *in vivo*. Although a single Comet assay was reported to show effects indicative of DNA damage, the Panel considers that this study does not provide substantive evidence of a genotoxic potential for stevioside, given methodological concerns and also the fact that similar findings were not seen in earlier studies in mice using steviosides of higher or lower purities. The Panel notes that steviol and some of its oxidative derivatives show clear evidence of genotoxicity *in vitro*, particularly in the presence of a metabolic activation system. However, studies of DNA damage and micronucleus formation in rats, mice and hamsters have shown that the genotoxicity of steviol is not expressed *in vivo* at doses of up to 8000 mg/kg bw. Given that the available toxicokinetic data indicate that free steviol is absent from the systemic circulation in humans or, at worst, present at very low (negligible) levels, any concern raised by the *in vitro* genotoxicity profile of steviol is fully addressed by the fact that the genotoxic potential of steviol is not expressed *in vivo*, and by the negative genotoxicity findings for steviol glycosides *in vitro* and *in vivo*.

The results of toxicological testing indicated that steviol glycosides are not genotoxic, carcinogenic, nor associated with any reproductive/developmental toxicity. The NOAEL in the 2-year carcinogenicity study in the rat was 2.5% stevioside (95.6% purity) equal to 967 mg stevioside/kg bw/day (corresponding to approximately 388 mg steviol equivalents/kg bw/day).

Single doses of 1000 mg steviol glycosides/person/day (97% rebaudioside A) (corresponding to approximately 330 mg steviol equivalents/day) did not affect glucose homeostasis and did not affect blood pressure in individuals with normal glucose tolerance or type-2 *diabetes mellitus*. Also repeated use for 16 weeks of 1000 mg rebaudioside A/person/day did not alter glucose homeostasis in individuals with type-2 *diabetes mellitus*. Blood pressure parameters were not significantly affected by oral intake of 1000 mg rebaudioside A/person/day for 4 weeks in individuals with normal and low systolic blood pressure. This daily dose corresponds to 16.6 mg of rebaudioside A/kg bw for a person weighing 60 kg and to approximately 5.5 mg steviol equivalents/kg bw/day.

Available data concerning anaphylaxis-like reactions by stevioside in children with atopic eczema do not, according to the Panel, raise concern regarding the potential for oral exposure to steviol glycosides to trigger anaphylactic reactions. Sparse *in vitro* and *in vivo* data indicate that stevioside may have immunostimulating effects and modulatory activities on inflammation. The Panel considered that immunostimulating and immunomodulating effects of steviol glycosides in cell lines and rodent models have not been demonstrated in a robust and reproducible way, which could enable them to be used as pivotal studies for risk assessment. However, these observations deserve more in-depth examination as, if they are confirmed, they may raise concern regarding the use of steviosides in some sub-groups of the population, particularly for individuals suffering from auto-immune diseases or inflammation of the gastrointestinal tract.

When considering the proposed maximum use levels (Tier 2), the mean dietary exposure to steviol glycosides expressed as steviol equivalents in European children (aged 1-14 years) ranged from 0.7 to 7.2 mg/kg bw/day, and from 3.3 to 17.2 mg/kg bw/day at the 95th percentile. The main contributors (>10% in all countries) to the total anticipated exposure to steviol glycosides, expressed as steviol equivalents, are soft drinks (11 to 58%) and desserts, including flavoured milk products (14 to 71%). Confectionery accounted for 11% of exposure in two countries. Dried potato granules and flakes and candied fruits and vegetables, mostardo di frutta accounted for 17 and 18% of exposure in one country.

Estimates reported for the UK adult population give a mean dietary exposure to steviol glycosides, expressed as steviol equivalents of 2.2-2.7 mg/kg bw/day and of 8.0-9.7 mg/kg bw/day for high level exposures (97.5th percentile). The main contributors to the total anticipated exposure to steviol glycosides expressed as steviol equivalents (>10 %) are soft drinks (37%) and beer, cider and perry (33%).

After considering all the data on stability, degradation products, metabolism and toxicology, the Panel establishes an Acceptable Daily Intake (ADI) for steviol glycosides, expressed as steviol equivalents, of 4 mg/kg bw/day based on application of a 100-fold uncertainty factor to the NOAEL for stevioside of 967 mg stevioside/kg bw/day (corresponding to approximately 388 mg steviol equivalents/kg bw/day) from a 2-year carcinogenicity study in the rat.

Conservative estimates of steviol glycosides exposures both in adults and in children suggest that it is likely that the ADI would be exceeded at the maximum use levels proposed by the petitioners.

KEY WORDS

Steviol glycosides, stevioside, rebaudioside A, Stevia.